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A new deep-sea scalpelliform barnacle, *Vulcanolepas buckeridgei* sp. nov. (Eolepadidae: Neolepadinae) from hydrothermal vents in the Lau Basin

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Abstract

The present study describes a new species of *Vulcanolepas* from the Lau Basin in the South Pacific. The basal angle of the tergum of *Vulcanolepas buckeridgei* **sp. nov.** is elevated from the capitular-peduncular margin at ~1/6 of the capitular height. The mandibles of *V. buckeridgei* **sp. nov.** are tridentoid; the cutting margins of the second and third teeth are long and each tooth possesses 18–20 sharp spines. The proximal segments of the anterior and posterior rami of cirrus I are protuberant and with dense, simple setae. DNA barcode sequences of *Vulcanolepas buckeridgei* **sp. nov.** are similar to *Vulcanolepas* sp. 1 collected from the Lau Basin (Herrera *et al.* 2015). *Vulcanolepas buckeridgei* is morphologically similar to *Vulcanolepas* 'Lau A' collected in the Lau Basin (Southward & Newman 1998). This suggests that *Vulcanolepas buckeridgei* **sp. nov.** is widespread in the Lau Basin.

Key words: Scalpelliformes, *Vulcanolepas,* hydrothermal vent taxa, Lau Basin

Introduction

Scalpelliform barnacles of the family Eolepadidae Buckeridge, 1983 are exclusively deep-sea hydrothermal vents species. Taxa within the subfamily Neolepadinae Yamaguchi, Newman & Hashimoto, 2004 have an eight plated capitulum (paired scuta, terga and medial latera, and the single carina and rostrum). The Neolepadinae is composed of two tribes and four genera. The tribe Ashinkailepadini Yamaguchi, Newman & Hashimoto, 2004 contains *Ashinkailepas* Yamaguchi, Newman & Hashimoto, 2004. The tribe Neolepadini Yamaguchi, Newman & Hashimoto, 2004 has *Leucolepas* Southward & Jones, 2003, *Neolepas* Newman, 1979 and *Vulcanolepas* Southward & Jones, 2003.

In the Lau Basin, two undescribed species of *Vulcanolepas*, 'Lau A' and 'Lau B,' were reported in Southward & Newman (1998) and a further *Vulcanolepas* sp. 1 was noted by Herrera *et al.* (2015). *Vulcanolepas* 'Lau A' is present in the central hydrothermal vent region, whilst *V.* 'Lau B' is located at the rim of the vents and sympatrically with *Eochionelasmus* Yamaguchi & Newman, 1990. The cirral setae on *Vulcanolepas* "Lau A" are very long, up to 14 times the length of an individual article on the cirrus (other neolepadids range from 6–9 times, see Southward & Newman 1998). Such long setae are coated by a white fuzz of filamentous sulphur bacteria. Southward & Newman (1998) concluded this *Vulcanolepas* "Lau A" to be ectosymbiotic with bacteria, farming the bacteria on the setae for food.

In the present study, two specimens of *Vulcanolepas* were collected in the Lau Basin in the South Pacific. These specimens featured long setae on the cirri and the setae were coated by white, filamentous bacteria and considered likely to be the same species as *Vulcanolepas* 'Lau A' in Southward & Newman 1998. From molecular analysis, this *Vulcanolepas* is also similar to *Vulcanolepas* sp. 1 in Herrera *et al.* (2015) and thus is an undescribed new species. This new species constitutes the fourth known species of *Vulcanolepas* and is described herein. The classification scheme for the Neolepadidae follows that of Buckeridge *et al.* (2013)

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Materials and methods

Specimen collection and morphological examination. The two *Vulcanolepas* specimens are housed in the collection of the Muséum national d'Histoire naturelle, Paris (France); they were collected in the Lau Basin during 2005 and 2006 (see material examined below) (Figure 1). The holotype was dissected and observed under light microscopy and descriptions of the capitular plates and arthropodal characters follow Buckeridge *et al.* 2013. Setal classification follows Chan *et al.* (2008).

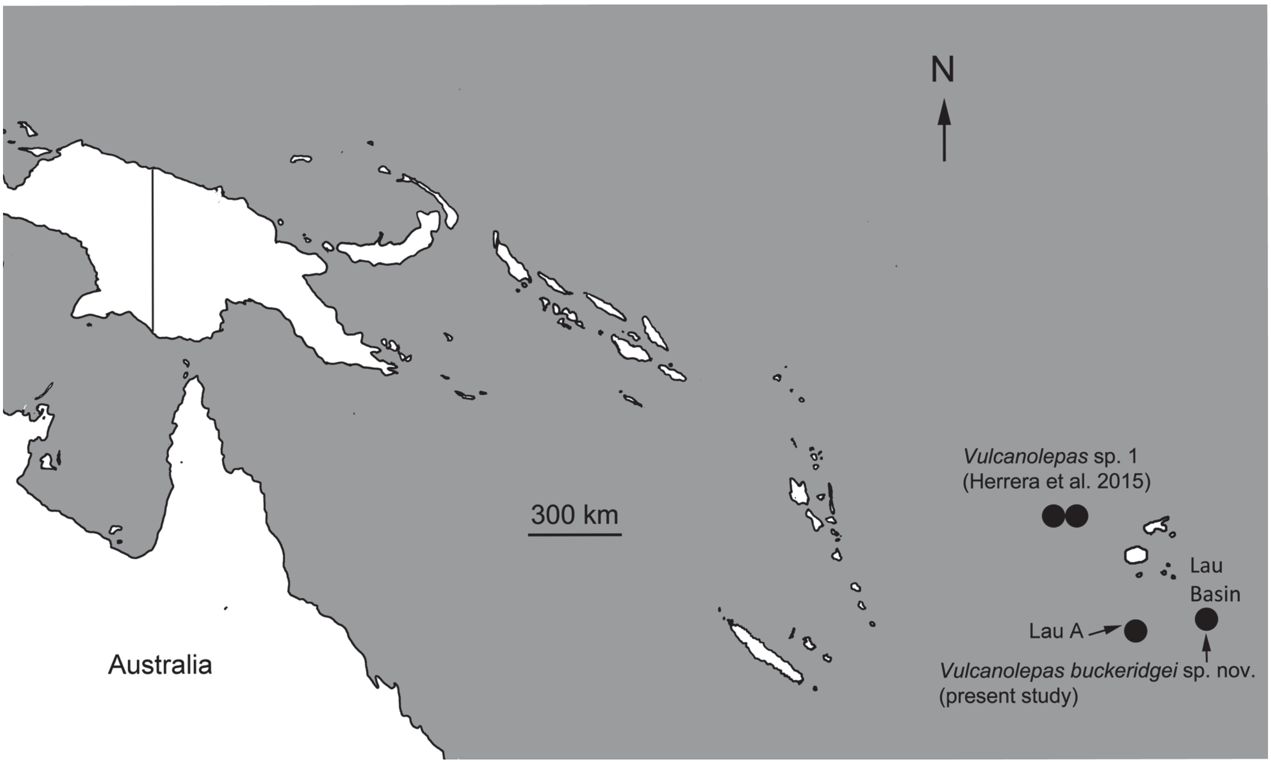

FIGURE 1. Sampling locations of *Vulcaolepas buckeridgei* **sp. nov.** and *Vulcanolepas* sp. 1 in Herrera *et al* (2015). Note DNA barcode sequences of *Vulcanolepas buckeridgei* and *V.* sp. 1 is similar, suggesting they are con-specific.

Molecular analysis. The mitochondrial COI gene (cytochrome *c* oxidase subunit I) was sequenced from two *Vulcanolepas* specimens. DNA was extracted from muscle tissue by using the commercial QIAamp Tissue Kit (QIAGEN). Partial sequences of mitochondrial COI gene were amplified using PCR universal primer set (LCO-1490/HCO-2198) for DNA barcoding (Folmer *et al.* 1994). Polymerase chain reaction (PCR) was performed using 25 µL of a solution containing 12.5 µL of Taq 2× Tools Taq Master Mix (AMPLIQON), 7.5 µL of deuterium-depleted water, 1 µL of each primer (5 µM), and 3 µL of the DNA template. The thermal regime entailed initialization for 4 mins at 94°C; denaturation, using 35 cycles for 0.5 min. at 94°C, 0.5 min. at 50°C, and 1 min. at 72°C; elongation for 10 mins at 72°C; and final extension at 12°C. The PCR products were observed on 1.5% agarose gel and the most intense products were used for Sanger sequencing. Successfully amplified PCR products were purified and sequenced using Genomics BioSci & Tech Ltd.

Phylogenetic analysis. Additional published sequences (81) from *Vulcanolepas* and related scalpellomorph taxa (Buckeridge *et al.* 2013; Herrera *et al.* 2015) were available from the National Center for Biotechnology Information (NCBI) for comparison (see Table S1). Sequences from *Neoverruca brachylepadoformis* Newman & Hessler, 1989 were included as outgroups in analysis as close to *Vulcanolepas scotiaensis* in Buckeridge *et al.* (2013). All sequences were aligned using the Clustal W (Larkin *et al.* 2007) plugin in Geneious R10 (Kearse *et al.* 2012). The aligned sequences resulted in 586 bp in the COI gene. The substitution model used was T92+G for the COI sequences, as tested in MEGA7 (Kumar *et al.* 2016). Maximum likelihood (ML) analyses, was conducted using MEGA7 with 1000 bootstrap replicates. Bayesian inference (BI) analysis was performed using *the* MrBayes 2.2 (Huelsenbeck & Ronquist 2001) plugin in Geneious R10. The BI analysis was run on the default setting for 1 million generations, applying one sample for every 100 generations. The first 25% of the sample tree was discarded as burn-in.

Systematics

Superorder Thoracica Darwin, 1854

Order Scalpelliformes Buckeridge and Newman, 2006

Family Eolepadidae Buckeridge, 1983

Subfamily Neolepadinae Yamaguchi, Newman & Hashimoto, 2004

Genus *Vulcanolepas* Southward & Jones, 2003

Vulcanolepas buckeridgei sp. nov.
(Figures 2–6)

Vulcanolepas Lau A. Southward & Newman, 2008: 260, fig. 1, fig. 2e, f, tab. 1
Vulcanolepas sp. 1. Herrera *et al.* 2015: fig. 2

Material examined. Holotype. MNHN-IU-2014-50277. Stn. Lau Basin 2005_JD-164, Lau Basin, Kilo Moana (22° 0' 0" S, 176° 0' 0" W, 26 June, 2005, depth 2628 m). Coll. Hourdez, S. Paratype. MNHN-IU-2014-5282 (damaged specimen). Stn. Lau Basin 2006_JD-235, Lau Basin, Kilo Moana (22° 0' 0" S, 176° 0' 0" W, 16 September, 2006, depth 2629 m). Coll. Hourdez, S.

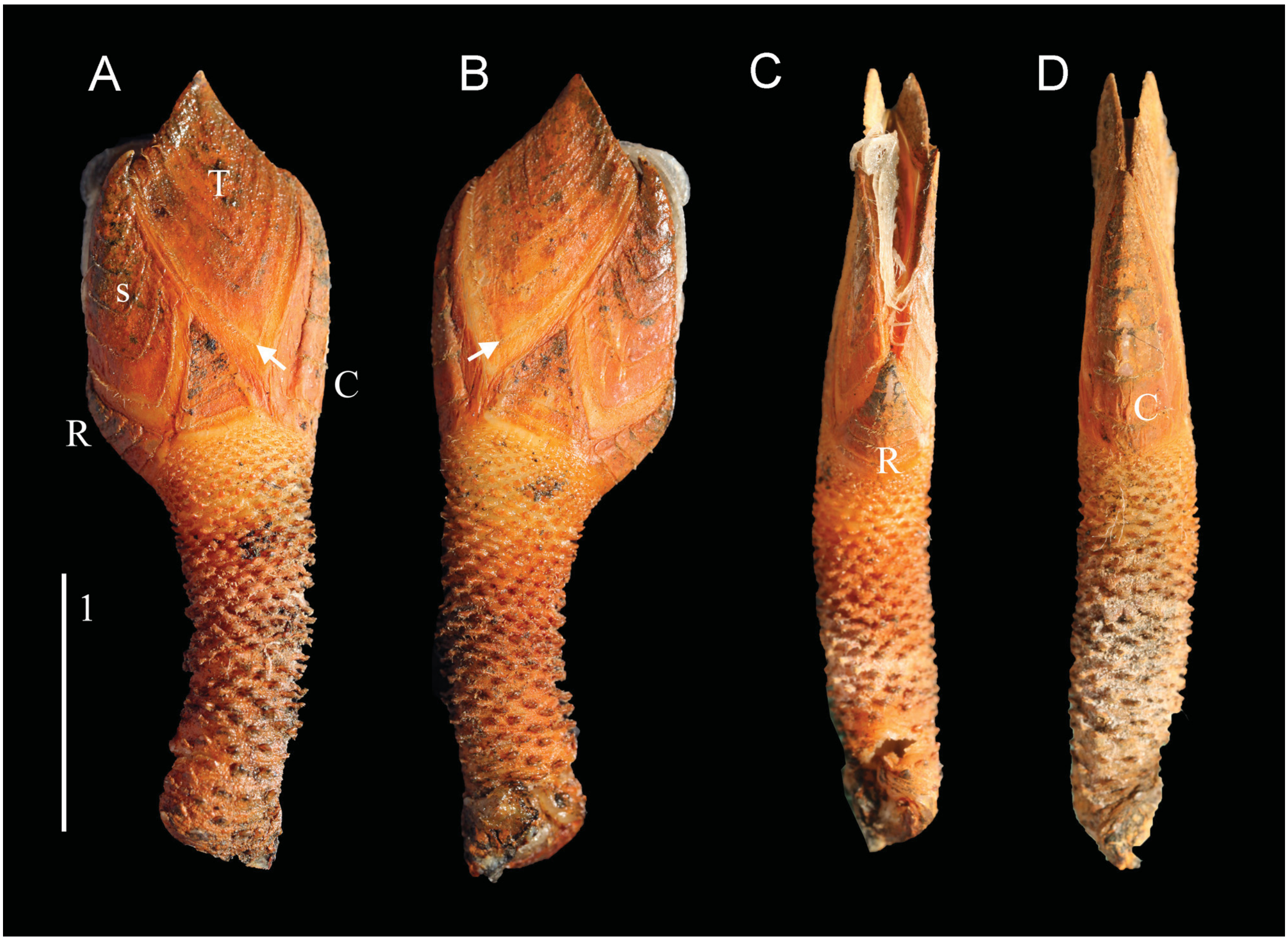

FIGURE 2. *Vulcanolepas buckeridgei* **sp. nov.** (holotype). A. Left view, B. Right view, C. Rostral view, D. Carinal view of whole specimens. T—tergum, S—scutum, R—Rostrum, C—Carina. White arrows indicate the basal angle of the tergum, which is elevated above the capitular-peduncular interface. Scale bars in cm.

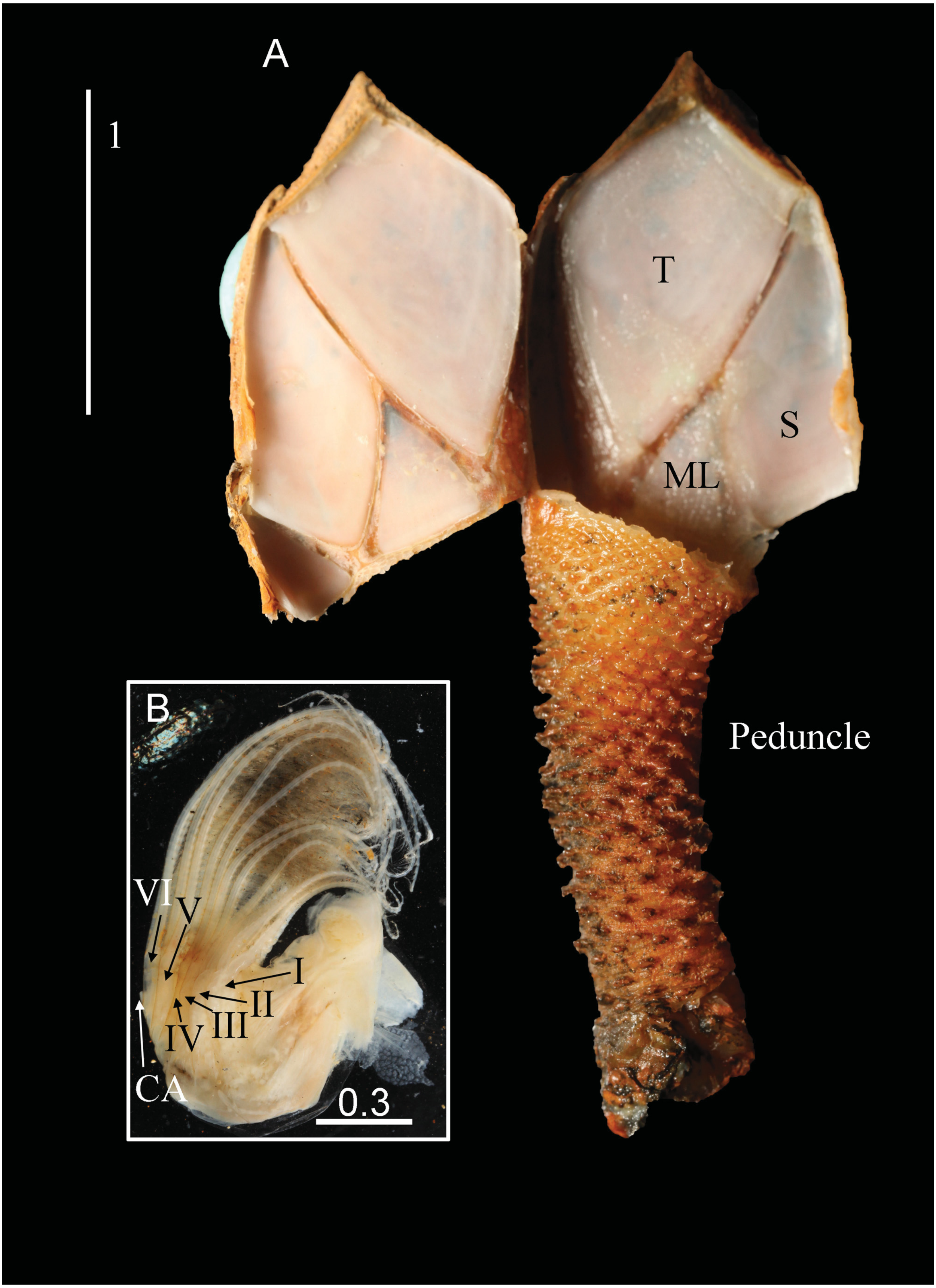

FIGURE 3. *Vulcanolepas buckeridgei* **sp. nov.** (holotype). Dissected individual showing the internal face of the capitulum (S—scutum, T—tergum, ML—medial latus). B. somatic body showing the six pairs of cirri (I–VI) and caudal appendage (CA). Note the six pairs of cirri are located close to each other, without the first pairs being separated from other pairs of cirri, as seen in most scalpellomorph species. Scale bars in cm.

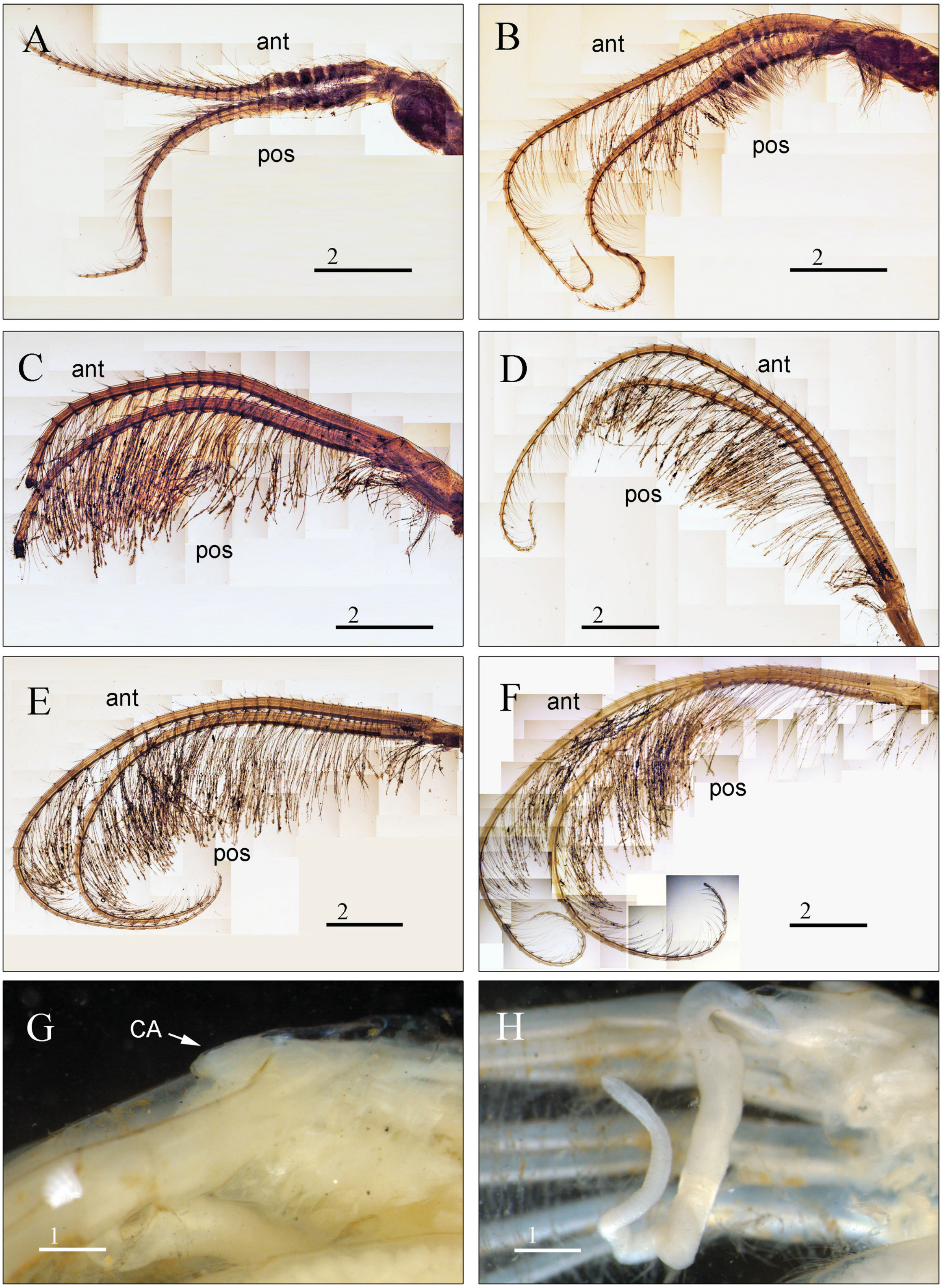

FIGURE 4. *Vulcanolepas buckeridgei* **sp. nov.** (holotype). A. Cirri I. B. Cirrus II. C. Cirrus III. D. Cirrus IV. E. Cirrus V. F. Cirrus VI. G. Caudal appendages (CA, indicated by arrow). H. Penis. Scale bars in µm. ant—anterior ramus, pos—posterior ramus.

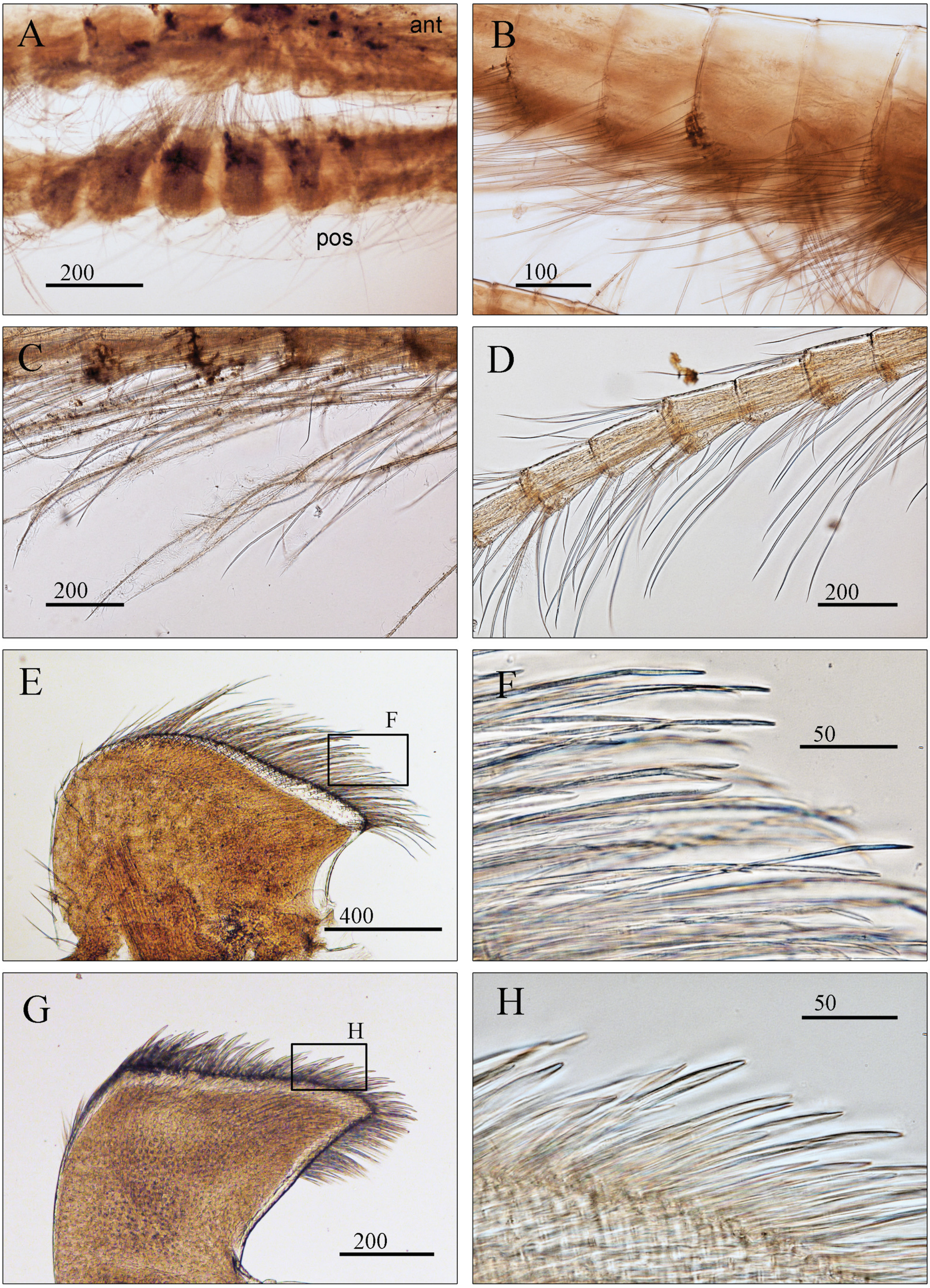

FIGURE 5. *Vulcanolepas buckeridgei* **sp. nov.** (holotype). A. magnified view of protuberant segments of the posterior (pos) and anterior rami (ant) of cirrus I. B. Simple setae of the anterior ramus of cirrus I. C. Serrulate setae of anterior ramus of cirrus I. D. Distal segments of anterior ramus of cirrus I E. Maxilla. F. Serrulate setae on the cutting margin of the maxillule. G. Maxillule. H. simple robust setae on the cutting edge of the maxillule. Scale bars in µm.

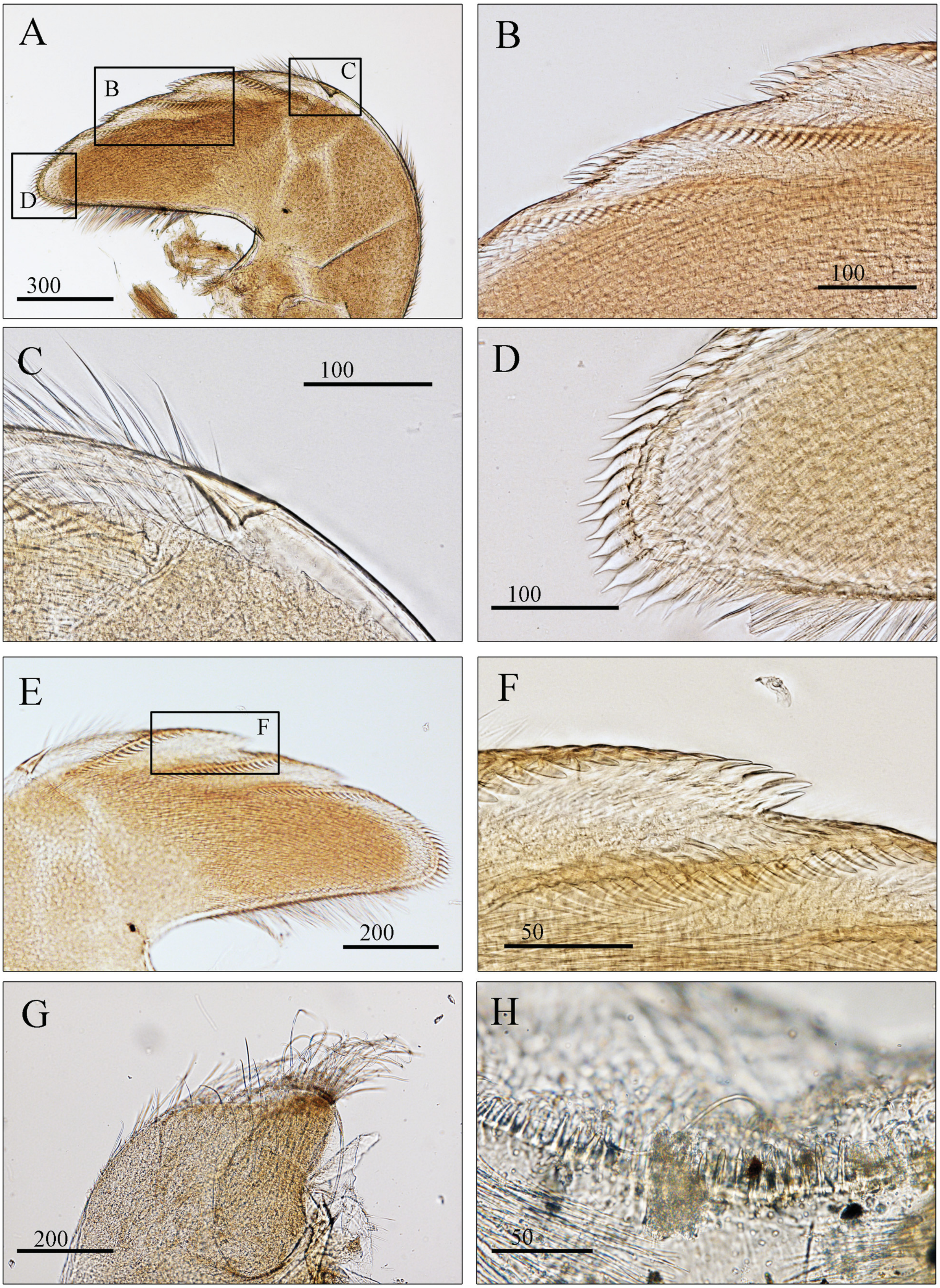

FIGURE 6. *Vulcanolepas buckeridgei* **sp. nov.** (holotype). A. Right mandible B. Serrated teeth on mandular cutting margin. C. Spine behind the first tooth. D. Inferior angle of mandible. E. Left mandible. F. Magnified view of serrated teeth on cutting margin. G. Mandibulatory palp. H. Labrum. Scale bars in µm.

Diagnosis. *Vulcanolepas* with basal angle of tergum elevated from the capitular-peduncular margin by ~1/6 of capitular height.Mandible tridentoid; cutting margin of second and third teeth long, each with 18–20 sharp spines. Proximal segments of anterior and posterior rami of cirrus I protuberant and with dense simple setae.

Description (based on holotype). Hermaphrodite capitulum higher than wide; 8 approximate plates, spaces between plates occupied by thin cuticular membranes. Carina umbo apical, slightly bowed, surface with 6 horizontal growth lines; height of carina 2/3 height of capitulum (Figure 2). Tergum quadrangular with single ridge extending from basal angle toapex; surface with V-shaped growth lines; basal angle of tergum slightly elevated from capitular-peduncular edge, at ~1/6 total height of capitulum (Figure 2). Scutum quadrangular, basal angle ~70°, located at capitular-peduncular edge; umbo apical; apex retro-curved (Figures 2, 3). Medial latus equilateral triangular, umbo apical, surface with 7 straight, horizontal growth lines. Rostrum triangular, 7 horizontal growth lines on surface.

Capitular:peduncular ratio ~1:1 (Figure 2). Peduncle with 23 scales/whorl just below capitular region, 24 scales/whorl at mid region of peduncle, scale width 0.87 mm (average from 3 scales), 0.73 mm (average from 3 scales) projecting from peduncle. Scales smaller at capitular region, becoming larger at basal region of peduncle. Internallly, capitular plates white, smooth (Figure 3A).

All six pair of cirri located close to each other, without first pair being separated (Figure 3B). Cirrus I with anterior and posterior rami similar in length, segments close to proximal region with width 2 x greater than length, rami becoming antenniform distally, with length 2 x greater than width (Figure 4A). Last 5 proximal segments of anterior and last 7 proximal of anterior ramus of cirrus I protuberant, bearing dense, simple setae (Figures 4A, 5A–D). Cirrus II with rami similar in length; proximal last 4–10 segments protuberant, with denser, simple setae (Figure 4B). Intermediate segments of cirrus II bearing 2 pairs of long setae and 3 pairs of short simple setae. Cirrus III with anterior and posterior rami similar in length, bearing very long setae, length of setae ~10 x length of individual segment; setae serrulate with very short, sparse setules, surface coated by filamentous bacteria (Figure 4C). Cirri IV–VI similar, with anterior and posterior rami similar length; distal segments of both rami with width two x greater than height (Figure 4D–F); setae long, ~10 x length of individual segment; intermediate segments of cirri IV to VI bearing 1 pair of short setae and 4 pairs of long setae. Caudal appendage unsegmented, short, blunt, length ~1/8 length of pedicel of cirrus VI (Figure 4G). Penis without basi-dorsal point, half length of cirrus VI (Figure 4H).

Maxilla subtriangular, with simple type setae on exterior margin (Figure 5E, F). Maxillule cutting edge straight, with dense, simple setae on cutting margin; interior margin slightly convex (Figure 5G, H). Mandible tridentoid; first tooth small, robust, sharp, separated from remainder (Figure 6A–F). Second and third teeth comb-shaped, 18–20 sharp spines on cutting edge. Cutting edge of second and third teeth long, each occupying 1/3 total length of mandible; inferior angle blunt, composed of series of sharp, large spines (Figure 6D). Mandibular palp elongated, with simple setae on tip and outer margin (Figure 6G). Labrum not bullate, with small, concave notch; single array of small, sharp teeth at notch (Figure 6H).

Etymology. Named in honour of Professor John S. Buckeridge (RMIT University, Melbourne, Australia) for his contribution to the taxonomy of hydrothermal vent stalked barnacles in the family Eolepadidae.

Distribution. Lau Arc Basin.

Remarks. The present study contributes the fourth species to *Vulcanolepas*. All four *Vulcanolepas* species have distinct morphological diagnostic characteristics. Both the mandible of *V. buckeridgei* **sp. nov.** and *V. parensis* Southward, 2005 are tridentoid, but the first tooth of *V. parensis* is much larger than the first tooth in *V. buckeridgei* **sp. nov.** In addition, *V. buckeridgei* **sp. nov.** has very long second and third mandibular teeth, each tooth taking 1/3 of the total length of the mandible, and each tooth bearing 18–20 spines on the cutting edge. In *V. parensis*, the length of the second and third teeth is ~1/7 of the total length of the mandible. The inferior angle of the mandible of *V. scotiaensis* contains dense setae, whilst the inferior angle of *V. buckeridgei* **sp. nov.** has several sharp spines, without any setae. The mandibles of *V. oshaei* have four teeth, differing from the other three *Vulcanolepas* species. Cirrus I of *V. buckeridgei* **sp. nov.** has protuberant segments at the basal region of both the anterior and the posterior rami. Protuberant segments are not reported in the other three species of *Vulcanolepas*.

Molecular results

Two COI sequences were amplified from two *V. buckeridgei* **sp. nov.** specimens (586 bp, Genbank Accession #

KY502196–KY502197) and five sequences of *Vulcanolepas* sp. 1 (Herrera *et al.* 2015) were retrieved from GenBank. Maximum-likelihood and Bayesian phylogenetic inference showed very strong branch support for each species except for *Neolepas* aff. *zevinaerapanuii* (Figure 7) which did not form a monophyletic group. *Vulcanolepas buckeridgei* **sp. nov.** formed a monophyletic group with *Vulcanolepas* sp. 1 (Herrera *et al.* 2015) with very strong support (bootstrap=98, posterior probability> 0.99). Kimura 2-parameter distance within *V. buckeridgei* **sp. nov.** ranged from 0.0–0.0029 while the genetic distance from other closely related *Vulcanolepas* species (*V. osheai* Buckeridge 2000 and *V. scotiaensis* Buckeridge, Linse & Jackson, 2013) ranged from 0.079–0.094 (see Table S2).

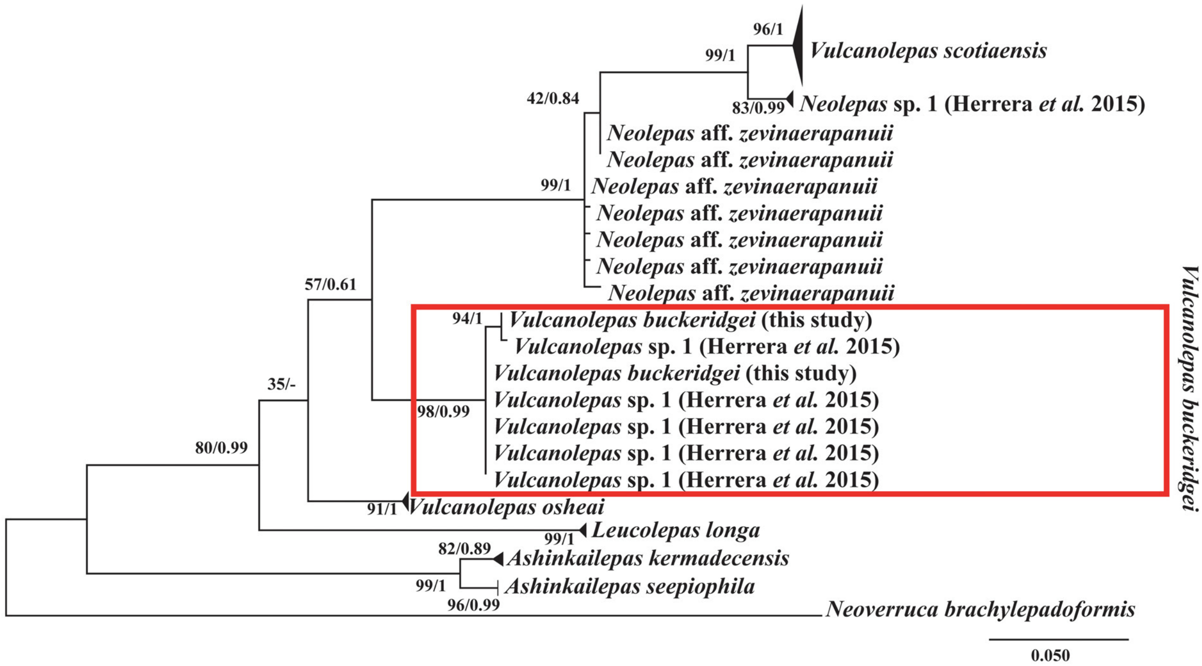

FIGURE 7. Genetic results showing that *Vulcanolepas buckeridgei* **sp. nov.** represents a single taxon with *Neoverruca brachylepadoformis* as outgroup in this study. Numbers at nodes are percentages of bootstrap support from maximum likelihood and posterior probabilities from Bayesian inference analysis (ML/BI).

Discussion

In the present study, *Vulcanolepas buckeridgei* **sp. nov.** is morphologically similar to *Vulcanolepas* 'Lau A' (Southward & Newman 2003). Figure 1A, B in Southward & Newman (2003) illustrates the external view of *Vulcanolepas* 'Lau A', which has a long stalk and the basal angle of tergum elevated ~1/4 total height of the capitulum above the capitular-peduncular margin. The basal angle of the tergum is elevated ~1/6 the total height of the capitulum, which is similar to *Vulcanolepas* 'Lau A'. Southward & Newman (2003) revealed the setal length in *Vulcanolepas* Lau A as 14 times the length of an individual segment, which differs from other described species of *Vulcanolepas*. In the present study, the length of the setae of cirri III–VI is ~10 times the length of an individual segment, which is close to the ratio determined from Southward & Newman (2003). These authors also illustrated the mandible of *Vulcanolepas* Lau A, which is tridentoid, with a single, small, first tooth and the second and third teeth filled with comb-like structures. The mandible illustrated in Southward & Newman (2003) is similar to *Vulcanolepas buckeridgei* **sp. nov.** in the present study.

From molecular analysis, the COI sequences of *Vulcanolepas buckeridgei* is almost the same as the sequence of *Vulcanolepas* sp. 1 in Herrera *et al.* (2015). The sampling points of *Vulcanolepas* sp. 1 were located at the Mata Uca Volcano at the Northwestern Slope vent site and Nantilis Marker 148 vent site in the NW part of the Lau Basin. The samples collected in the present study were located at the central region of the Lau Basin. *Vulcanolepas* 'Lau A' from Southward & Newman (1998) was collected from the BIOLAU expedition in the Lau Basin (see Table 2 in

Desbruyeres *et al.* 1994) from the Hine Hina site (22° 32' S, 176° 43' W), which is close to the type locality of *V. buckeridgei* **sp. nov.** This suggests that *Vulcanolepas buckeridgei* **sp. nov.** is widely distributed in the Lau Basin. Reviewing the distribution record of *Vulcanolepas*, it appears that *Vulcanolepas* has a distinct distribution amongst deep-sea basins. *Vulcanolepas scotiaensis* is recorded in the Scotia Sea off Antarctica, *V. parensis* from the Pacific Antarctica-Ridge, *V. oshaeai* is present on the Kermadec Ridge in New Zealand waters and *V. buckeridgei* **sp. nov.** occurs in the Lau Basin in the South Pacific.

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TABLE S1. Accession numbers for sequences retrieved from GenBank.

Taxon ID	GenBank Acc.
Ashinkailepas kermadecensis	KP295061
Ashinkailepas kermadecensis	KP295053
Ashinkailepas kermadecensis	KP295048
Ashinkailepas kermadecensis	KP295040
Ashinkailepas kermadecensis	KP295019
Ashinkailepas kermadecensis	KP295001
Ashinkailepas seepiophila	KP295091
Ashinkailepas seepiophila	KP295090
Ashinkailepas seepiophila	KP295069
Ashinkailepas seepiophila	KP295046
Ashinkailepas seepiophila	KP295031
Ashinkailepas seepiophila	KP295028
Ashinkailepas seepiophila	KP295022
Leucolepas longa	KP295027
Leucolepas longa	KP295073
Leucolepas longa	KP295076
Leucolepas longa	KP295082
Leucolepas longa	KP295083
Leucolepas longa	KP295085
Neolepas aff. *zevinaerapanuii*	KP295098
Neolepas aff. *zevinaerapanuii*	KP295084
Neolepas aff. *zevinaerapanuii*	KP295067
Neolepas aff. *zevinaerapanuii*	KP295063
Neolepas aff. *zevinaerapanuii*	KP295060
Neolepas aff. *zevinaerapanuii*	KP295055
Neolepas aff. *zevinaerapanuii*	KP295007
Neolepas sp. 1	KP295004
Neolepas sp. 1	KP295030
Neolepas sp. 1	KP295032
Neolepas sp. 1	KP295047
Neolepas sp. 1	KP295062
Neolepas sp. 1	KP295064
Neolepas sp. 1	KP295089

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TABLE S1. (Continued)

Taxon ID	GenBank Acc.
Neoverruca brachylepadoformis	AB195606
Vulcanolepas osheai	KP295005
Vulcanolepas osheai	KP295008
Vulcanolepas osheai	KP295026
Vulcanolepas osheai	KP295034
Vulcanolepas osheai	KP295036
Vulcanolepas osheai	KP295049
Vulcanolepas osheai	KP295056
Vulcanolepas osheai	KP295094
Vulcanolepas scotiaensis	KP295097
Vulcanolepas scotiaensis	KP295078
Vulcanolepas scotiaensis	KP295068
Vulcanolepas scotiaensis	KP295058
Vulcanolepas scotiaensis	KP295057
Vulcanolepas scotiaensis	KP295052
Vulcanolepas scotiaensis	KP295050
Vulcanolepas scotiaensis	KP295045
Vulcanolepas scotiaensis	KP295042
Vulcanolepas scotiaensis	KP295039
Vulcanolepas scotiaensis	KP295037
Vulcanolepas scotiaensis	KP295035
Vulcanolepas scotiaensis	KP295018
Vulcanolepas scotiaensis	KP295014
Vulcanolepas scotiaensis	KP295013
Vulcanolepas scotiaensis	KF739820
Vulcanolepas scotiaensis	KF739821
Vulcanolepas scotiaensis	KF739822
Vulcanolepas scotiaensis	KF739823
Vulcanolepas scotiaensis	KF739824
Vulcanolepas scotiaensis	KF739825
Vulcanolepas scotiaensis	KF739826
Vulcanolepas scotiaensis	KF739827
Vulcanolepas scotiaensis	KF739828
Vulcanolepas scotiaensis	KF739829
Vulcanolepas scotiaensis	KF739830
Vulcanolepas scotiaensis	KF739831
Vulcanolepas scotiaensis	KF739832
Vulcanolepas scotiaensis	KF739833
Vulcanolepas scotiaensis	KF739834
Vulcanolepas scotiaensis	KF739835
Vulcanolepas scotiaensis	KF739836
Vulcanolepas scotiaensis	KF739837

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TABLE S1. (Continued)

Taxon ID	GenBank Acc.
Vulcanolepas scotiaensis	KF739838
Vulcanolepas sp. 1	KP295080
Vulcanolepas sp. 1	KP295051
Vulcanolepas sp. 1	KP295041
Vulcanolepas sp. 1	KP295033
Vulcanolepas sp. 1	KP295009
Vulcanolepas buckeridgei **sp. nov.**	KY502196
Vulcanolepas buckeridgei **sp. nov.**	KY502197

TABLE S2. Cytochrome c oxidase subunit I (COI) pairwise distance (K2P distances) between species. There were a total of 586 bp in this dataset and analysis were conducted in MEGA 7.

	1	2	3	4	5	6	7	8	9
1. *Ashinkailepas kermadecensis*									
2. Ashinkailepas seepiophila	0.024								
3. *Leucolepas longa*	0.153	0.152							
4. *Neolepas* aff. *Zevinaerapanuii*	0.162	0.171	0.129						
5. *Neolepas* sp. 1	0.166	0.170	0.126	0.060					
6. *Neoverruca brachylepadoformis*	0.176	0.183	0.213	0.184	0.197				
7. *Vulcanolepas osheai*	0.134	0.137	0.113	0.090	0.110	0.169			
8. *Vulcanolepas scotiaensis*	0.164	0.166	0.136	0.061	0.028	0.198	0.110		
9. *Vulcanolepas buckeridgei* **sp. nov.**	0.144	0.153	0.111	0.082	0.095	0.185	0.079	0.094	